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Transcriptional and Posttranscriptional Regulation of Exogenous Human Beta Interferon Gene in Simian Cells Defective in Interferon Synthesis

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We determined that the defect in beta interferon induction in Vero cells is due to the absence of the simian beta interferon (IFN-β) gene. Nevertheless, the human IFN-β gene or a hybrid gene, in which the human IFN-β promoter-regulatory region directs expression of the chloramphenicol acetyltransferase gene (pIFN-CAT), could be induced in transfected Vero cells, and these cells also regulated IFN-β mRNA (but not pIFN-CAT mRNA) posttranscriptionally. These results indicate that the instability in the human IFN-β gene is coded for by the coding or 3'-end region of IFN-β mRNA and that the human IFN-β gene is regulated in Vero and human cells in an identical manner.

DNA sequences complementary to human beta interferon have been detected in all mammals examined and in nonmammalian vertebrates ranging from birds to bony fish (30), and interferon synthesis can be induced by viral infection in most cell lines examined. There are only a few cell types not able to induce interferon synthesis as a response to viral infection. These are mainly undifferentiated cells such as neuroblastoma or embryonal cells (1, 3, 11). These cells are also generally not sensitive to the antiviral effect of exogenous interferon; both of these blocks can be released after differentiation. A fibroblastic cell line that will not produce interferon after either viral infection or treatment with double-stranded RNA is a continuous line derived from African green monkey kidney-Vero cells. However, this cell line is sensitive to the antiviral effect of interferon and has been used extensively for assay of human interferon in medium which contains interferon inducers. The nature of the defect in interferon synthesis in Vero cells is not known. Previous studies have shown that somatic cell hybrids between either mouse L cells and Vero cells (6) or human lymphocytes and Vero cells (14) produced mouse and human interferons, respectively, but not primate interferon. These results suggest that the lack of interferon synthesis in Vero cells is not due to the presence of a repressor that interferes with interferon induction. Whether the defect in interferon inducibility is due to deletion or mutation of the structural gene for beta interferon or whether the Vero cells lack the cell type-specific *trans*-acting factors essential for interferon induction is not known.

The aim of the present study was to examine on the molecular level the nature of the defect in interferon synthesis in Vero cells and to determine whether Vero cells would regulate the human beta interferon gene in a manner similar to that observed in human cells. Since our previous results showed that in human fibroblasts both viral infection and poly(rI · rC) induce predominantly expression of the beta interferon gene, we focused our study on beta interferon gene expression. We confirmed the lack of synthesis of biologically active interferon after induction of Vero cells and extended this observation to the mRNA level using CV-1 monkey cells as a positive control (Fig. 1). Whereas in induced human fibroblasts and CV-1 cells we detected properly initiated beta interferon mRNA, no beta interferon mRNA was found in induced Vero cells. The reason for the lack of simian beta interferon mRNA and protein became apparent upon analysis of Vero cell genomic DNA (24) by Southern hybridization (26) (Fig. 2). In human fibroblasts (MG-63 cells) and CV-1 monkey cells, we detected sequences complementary to the human beta interferon gene, but no hybridization of the human beta interferon cDNA probe (21) was detected with genomic DNA from Vero cells (Fig. 2A). As a control, we hybridized the same DNA with beta actin probe; all three cell lines contained sequences complementary to the chicken beta actin gene (Fig. 2B), indicating that failure to detect a beta interferon gene in Vero DNA was not due to the absence of hybridizing DNA. These results show that the primate beta interferon gene is not present in the genomic DNA of Vero cells. Thus, the defect in interferon synthesis in these cells is the deletion of the structural gene for beta interferon.

To determine whether Vero cells contain the *trans*-acting factors necessary for beta interferon induction, we constructed a hybrid plasmid in which expression of the chloramphenicol acetyltransferase (CAT) gene was regulated by the promoter region of the human beta interferon gene (pIFN-CAT) (Fig. 3). Expression of this hybrid gene was initially tested after transfection into Ltk^- cells, since we and others have previously shown that in these cells the transfected beta interferon gene can be induced both by viral infection and poly(rI · rC) (J. D. Mosca and P. M. Pitha, unpublished data) (4, 12, 15, 20, 28, 31). To test the specificity of induction, the Ltk^- cells were also transfected with plasmids in which the CAT gene was inserted behind the herpes simplex virus type 1 (HSV-1) thymidine kinase promoter (pTK-CAT) (19) and the simian virus 40 early promoter (pSV2CAT) (10). The plasmids were transfected by the calcium chloride method described previously (23) modified by sodium butyrate enhancement (9). Transfected cells were either mock infected or infected with either Newcastle disease virus (NDV) or HSV, and 16 h after infection cell extracts were assayed for CAT activity. All three hybrid

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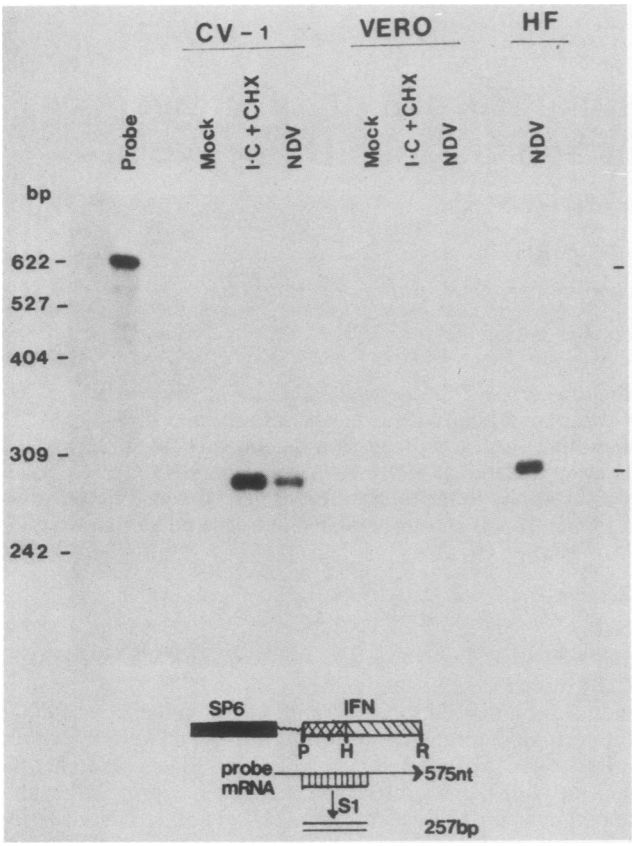

FIG. 1. S1 analysis of endogenous beta interferon RNA. Total RNA from either induced or uninduced cells (10 μg from CV-1 and Vero cells, 2 μg from human fibroblasts [HF]) was hybridized to an excess (5×10^5 cpm) of a 575-nucleotide (nt)-long complementary-strand SP6 RNA probe (2×10^8 cpm/μg of DNA), followed by S1 nuclease digestion, fractionation on a 5% polyacrylamide-urea sequencing gel at 30 mA, and autoradiography (4 h). The probe lane contains undigested probe (575 nt). The construction of the probe and the fragment generated, after RNA hybridization, by RNA and S1 treatment are shown in the diagram below the figure. Indicated lengths are in nucleotides. R indicates the *Eco*RI site at −285; H indicates the *Hin*cII site at +50; P indicates the *Pst*I site at +275. The wavy line indicates the 15-nt region of the probe that is not complementary to the interferon sequence. The hatched area represents the human beta interferon promoter, and the cross-hatched area represents a portion of the interferon 5′ coding region. CHX, Cycloheximide; bp, base pairs.

plasmids were expressed constitutively in Ltk$^-$ cells (Fig. 4A). Expression of pIFN-CAT and pTK-CAT was 100-fold and 30-fold lower than expression of pSV2CAT. Infection with NDV increased expression of pIFN-CAT by 12-fold but inhibited expression of pTK-CAT. Infection with HSV increased expression of pTK-CAT and pIFN-CAT fivefold and threefold respectively. Since in human cells interferon synthesis can be induced with NDV but not with HSV infection, these data indicate that the observed induction of pIFN-CAT by NDV shows similar specificity. Expression of pSV2CAT was not increased by viral infection (data not shown).

Expression of pIFN-CAT in Vero cells is shown in Fig. 4B. Transfected Vero cells were infected with NDV or induced with poly(rI · rC) (50 μg/ml) in the presence of cycloheximide (50 μg/ml) for 4 h, after which the cycloheximide was removed by repeated washings to restore protein synthesis, and CAT activity was determined 16 h later. The constitutive expression of pIFN-CAT, in Vero cells, is negligible (Fig. 4B). Induction with NDV or poly(rI · rC) enhanced pIFN-CAT expression 16- and 56-fold, respectively. These results show that the human beta interferon promoter can be induced in Vero cells and that Vero cells are not deficient in *trans*-acting factors required for beta interferon expression.

We have shown previously that in human cells induced with poly(rI · rC) there are two levels of regulation of beta interferon gene expression. Activation of transcription and alteration of beta interferon alter mRNA stability (22). Whereas activation of transcription occurs in the absence of cellular protein synthesis, beta interferon mRNA degradation is coupled to cellular protein synthesis. It was therefore of interest to determine whether human beta interferon mRNA is also posttranscriptionally regulated in Vero cells. Vero cells were cotransfected either with the pIFN-CAT plasmid or the cloned human beta interferon gene (pIFR) (31) together with the plasmid carrying the neomycin gene (pSV2neo) (27), and colonies resistant to G418 were selected. Transfected cultures were induced for 4 h with poly(rI · rC) in the presence of cycloheximide. The RNA was isolated (5) at the end of the induction period or 2 or 4 h after cycloheximide removal and analyzed by S1 nuclease mapping (2, 16, 29) using a single-strand SP6 promoter (17)-directed RNA probe. Analysis of RNA isolated from cells transfected with pIFN-CAT shows the presence of correctly initiated CAT mRNA, which persisted in these cells for at least 4 h after cycloheximide removal (Fig. 5B). The RNA isolated from Vero cells transfected with a genomic beta interferon clone (pIFR) shows the presence of beta interferon mRNA only in cells induced by poly(rI · rC) in the presence of cycloheximide; once cycloheximide was removed, beta interferon mRNA rapidly degraded (Fig. 5A). Thus, in transfected Vero cells as in human cells, induced

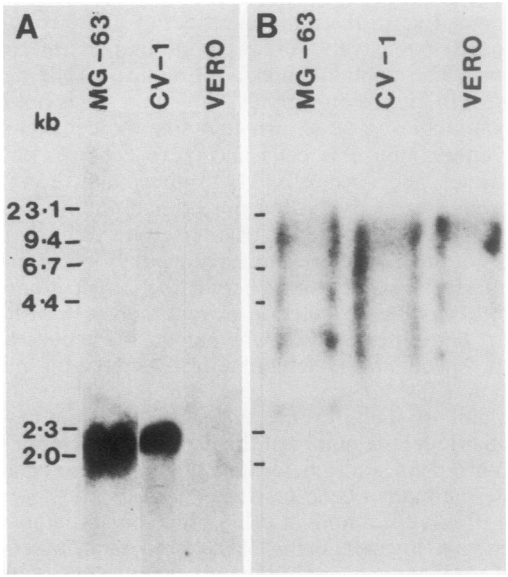

FIG. 2. Southern blot analysis of sequences complementary to the human beta interferon gene in human MG osteosarcoma, Vero, and CV-1 monkey cells. High-molecular-weight DNA was restricted with *Eco*RI, electrophoresed through a 0.8% agarose gel, transferred to nitrocellulose, and hybridized to either human beta interferon cDNA (A) or chicken beta actin (B) nick-translated probes. Size markers (in kilobases [kb]) are indicated on the left.

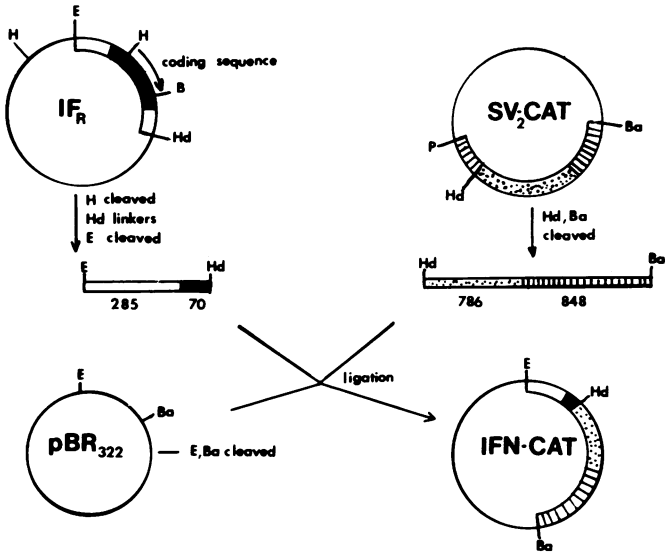

FIG. 3. Construction of the pIFN-CAT plasmid. The diagram illustrates the steps involved in placing the human beta interferon promoter-regulatory region in front of the chloramphenicol acetyltransferase (CAT) gene. The pIFR plasmid containing the beta interferon gene with its 5′ and 3′ flanking regions was digested with *Hin*cII. The *Hin*cII site is located 3 base pairs (bp) upstream from the translational initiation site, and therefore the hybrid contains 70 bp of the 5′ nontranslated region of beta interferon mRNA. The 700-bp-long fragment was separated by electrophoresis in low-melting-point agarose, and *Hin*dIII linkers were ligated to this fragment. After cleavage with *Eco*RI, the isolated human beta interferon promoter (350 bp) was ligated together with the 1,630-bp-long *Hin*dIII-*Bam*HI (CAT coding region with polyadenylation signals from simian virus 40) fragment obtained from pSV2CAT and the *Eco*RI-*Bam*HI fragment of pBR322. The translational initiation codon for the CAT protein represents the first initiation codon in the pIFN-CAT fusion gene. The solid bars represent the human interferon beta cDNA sequence. The open bars represent the 5′ and 3′ regions of the human beta interferon gene. The striped bars represent simian virus 40 sequences. The dotted bars represent the CAT coding sequence. The letters H, E, B, Hd, Ba, and P represent the restriction enzymes *Hin*cII, *Eco*RI, *Bgl*II, *Hin*dIII, *Bam*HI, and *Pvu*II, respectively.

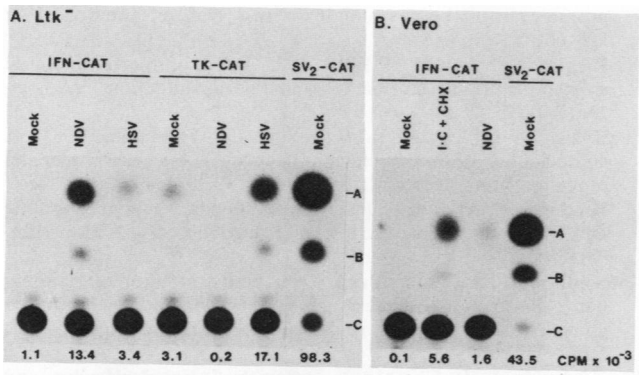

FIG. 4. Transient expression of CAT activity in either Ltk$^-$ (A) or Vero (B) cells. The hybrid plasmids (10 μg) were transfected in parallel, glycerol shocked, and then either mock infected, treated with poly(rI · rC) (50 μg/ml)-cycloheximide (CHX) (50 μg/ml) for 4 h and subsequently released, or infected with NDV (NJ-LaSota; multiplicity of infection = 10) or HSV-1 (MPcl-20; multiplicity of infection = 3). Cell cultures were terminated 16 h after induction, extracts were prepared as previously described (18), and the acetylated products of chloramphenicol were separated by chromatography and visualized by autoradiography. The radioactivity in counts per minute (cpm) associated with the 3-acetylchloramphenicol products is given for quantitative comparison.

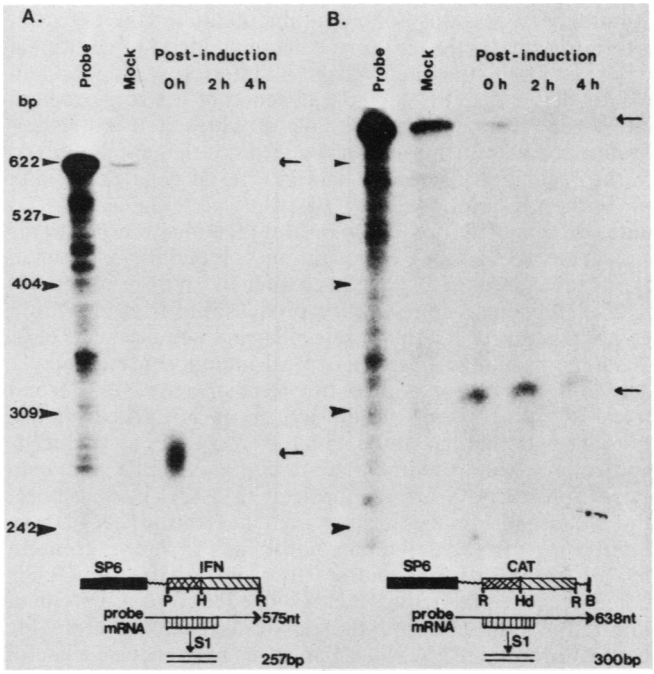

FIG. 5. S1 analysis of RNA isolated from Vero cell lines containing the pIFN-CAT hybrid and the genomic human beta interferon clone (pIFR). Vero cells were transfected with either (A) the human beta interferon gene or (B) the pIFN-CAT hybrid in the presence of the neomycin gene as described recently (18). The transfected cells were selected by growth in G418 (1 mg/ml) and tested for inducibility of CAT activity and synthesis of human beta interferon. Two highly inducible clones (VNβCATcl-25 and VNβIFNcl-13) were selected for further studies. Total RNA (10 μg) (A) or poly(A)$^+$-selected RNA (5 μg) (B) was hybridized to 5×10^5 cpm of the ^{32}P-labeled RNA probe synthesized from the SP6 promoter (*Eco*RI fragment of pIFN-CAT or *Eco*RI-*Pst*I fragment of pIFR). Hybridization and S1 nuclease digestion were done as previously described (2, 29). The protected fragments were analyzed on a 5% polyacrylamide-urea sequencing gel (16) at 30 mA and autoradiographed. Construction of the probe and the size of the protected fragment generated after hybridization with RNA and S1 treatment are shown in the diagram below each figure. The letters H, Hd, P, and R represent the restriction enzymes *Hin*cIII, *Hin*dIII, *Pvu*II, and *Eco*RI, respectively. The probe lane contains undigested probe. Indicated lengths are in nucleotides (nt). The wavy line indicates the region of the probe that is not complementary to IFN or CAT sequences. The hatched area represents the human beta interferon promoter, and the crossed area represents a portion of either the interferon or CAT 5′ coding region. bp, Base pairs.

human beta interferon mRNA is not stable and is quickly degraded, whereas the half-life of CAT mRNA under the same conditions is much longer. Since the pIFN-CAT construct contains both the promoter region and nontranslated 5′ sequences, these results indicate that the stability of beta interferon mRNA is specified by the coding region, the 3′ untranslated region, or both. Additional studies are in progress to determine which segment of beta interferon mRNA is recognized by the shutoff (degradation) mechanism.

Initially, it was our hope that elucidation of the defect in interferon production in Vero cells would lead to additional information on the mechanism of interferon induction in mammalian cells. Instead, the absence of a structural beta interferon gene in this cell line provides a useful assay system for identification of structural *cis* elements involved in the regulation of human beta interferon gene expression on both transcriptional and posttranscriptional levels. To date, the majority of studies on the identification of the *cis* sequences regulating expression and inducibility of human type I interferon genes have been done in a mouse system (7, 8, 25). However, the regulation processes of beta interferon genes in mouse and human cells differ in several aspects (13). Whereas in human fibroblasts viral infection preferentially activates expression of the beta interferon genes (21, 22) and leads to the synthesis of beta interferon mRNA, in mouse fibroblasts both alpha and beta interferon genes are induced, and approximately equimolar amounts of alpha and beta interferon mRNAs are synthesized (13, 32). Furthermore, the shutoff mechanism which regulates the levels of beta interferon mRNA in human fibroblasts is not present in mouse fibroblasts. In contrast to mouse cells, Vero cells contain the *trans*-acting factors needed for human beta interferon induction and the posttranscription factors for beta interferon mRNA shutoff and thus may provide a useful system for studies of transcriptional and posttranscriptional regulation of the human beta interferon gene.

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